1960

The effect of splenectomy on isohemagglutinin production in the rabbit

Thomas Joseph Lau
Yale University

THE EFFECT OF SPLENECTOMY ON ISOHEMAGGLUTININ PRODUCTION IN THE RABBIT

by

Thomas Lau

A Thesis

Presented to the Faculty of the School of Medicine of Yale University

In Candidacy for the Degree of

Doctor of Medicine

Department of Surgery

1960

My deep and heartfelt thanks to my advisor, Dr. Mark Hayes, and all the many others who helped me.

My deep and heartfelt thanks to my advisor, Dr. Mark Hayes,

and all the many others who helped me.

A great deal of research concerning the site of antibody formation has been carried out.[1] Some studies implicate the lymphoidal tissue and lymphocyte;[2,3] others the plasma cell,[4] still others the splenic cells that later develop into plasma cells.[5] However, it is not likely that the capacity to produce antibodies is localized to a single type of cell, but rather is possessed by cells that are widely scattered throughout the body,[6] and whose response is determined by the nature, quantity and route of administration of the antigen.[7,8]

Despite the fact that the clinical reports of increased susceptibility to infections in splenectomized patients are inconclusive and conflicting;[9,10,11,12] the evidence that the spleen is an important site of antibody formation is great;[13-25] and in 1950, D.A. Rowley[26] showed that a splenectomized man responds with very little circulating antibody to particulate erythrocytic antigen (1.0 ml. of a 2.0 percent suspension of sheep red cells) introduced intravenously. Since the hemolytic manifestations of erythroblastosis foetalis have been shown[27] to be due to transfer across the placenta of maternal isoantibodies that are specific for certain antigens, notably those of the Rh system, inquiries as to the role of the spleen in the production of circulating isoantibodies seemed practical.

Erythroblastosis foetalis occurs naturally in man, horses,[28] and donkeys,[29] while the hemolytic aspects of the disease have been produced experimentally in dogs[30] and pigs.[31] Furthermore, doe rabbits injected first subcutaneously with incompatible rabbit red cells incorporated in Freund's adjuvant[32] and then intravenously with the same saline suspended red cells develop significant isoantibody titers. If these does then mate with bucks bearing the appropriate incompatible iso-agglutinogen, young will be born not only with the hematologic disturbance, but also with the main histologic features of the condition.[33] Now, if the spleen were a determining factor in the production of circulating isoantibody in this disease, then a splenectomized rabbit should produce little or none of it. In that event splenectomy might be effective therapy for some Rh negative mothers who had delivered erythroblastotic babies and desired a normal child.[26]

However, the difficulty in producing adequate isohemagglutinins in the rabbit is well known and almost all workers employ non-specific antigen adjuvants such as B.C.G. or Freund's preparation of heat killed tubercle bacilli to obtain faster and higher titers.[34-36]

We now know that the large granulomata developed at the injection sites of the antigen-adjuvant emulsions contribute a significant part of the total resulting antibody.[6,7] Since I planned to evaluate splenic function, these interfering

granulomatous antibody sources were unacceptable, and I chose to follow the evidence[36] suggesting that isohemagglutination titers run on sera drawn three times a week during and after the immunization program would demonstrate significant differences between the splenectomized and the control rabbit groups.

MATERIALS AND METHODS

Rabbits: adult male and female Cottontail, New Zealand, and mixed breed rabbits were purchased from three different dealers in and aroundNew Haven, housed two in a cage, and fed Purina chow and water ad lib.

Blood Typing Antisera: generously supplied by Dr. J.J. Griffitts[37] and identified as anti-G and anti-g (following Kellner's symbols).[38,39] This serum, which was obtained from one of the twelve rabbits given repeated intravenous injections of red blood cells from rabbits of a presumably different strain, was found by Griffitts to contain an antibody that agglutinated the suspended red cells of some rabbits and not those of others, and is well accepted in this field.

Isotonic Alsever's Solution (Modified):

glucose	2.05 grams
sodium citrate	0.80 grams
sodium chloride	0.42 grams
distilled water q.s. ad.	100 ccs.

adjust to pH 6.1 with 5 per cent citric acid

Modified Veronal Buffer Solution:

sodium chloride	83.8 grams
sodium bicarbonate	2.52 grams
sodium barbital	3.00 grams
acid barbital	4.60 grams
magnesium chloride solution	0.2 molar 25 ccs.

dissolve acid in 500 milliliters of hot distilled water, add the other components to this solution, cool to room temperature and dilute to ten liters for use.

In order to avoid the spontaneous agglutination which occurs about half the time when rabbit red cells are suspended in saline,[34-40] this solution was modified to contain 5 per cent of normal serum from a Gg rabbit when finally used.

Blood Typing of Rabbits: Whole blood was drawn from a central ear vein and mixed with an equal volume of Alsever's citrate-saline solution. One drop of this erythrocytic-citrate suspension was placed on each of two clean glass slides, to one slide a single drop of anti-G typing serum was added and to the other slide one drop of anti-g serum was added. Patterns of agglutination under a hand lens were recorded immediately as zero to four plus.

Iso-Immunization: A single type GG animal (animal #110) supplied all the red cells used for the immunizations and titrations of iso-antibodies. Its blood was collected by cardiac puncture into an equal volume of Alsever's solution, centrifuged at 2,000 r.p.m. for five minutes and washed three times in the veronal buffer. After drawing blood by cardiac puncture for later antibody titers, one cubic centimeter of a 50 per cent suspension of these thrice washed red cells in buffer was injected into the central ear vein of each of the ten type gg experimental animals. This dose was repeated three times a week for three weeks.

Splenectomy: Five type gg rabbits were splenectomized under intravenous barbituate anesthesia (pentobarbital 15 mgms. per pound of body weight). A scissor cut was made through all the layers of the abdominal wall, induding the peritoneum, to form a left pararectus incision of moderate length. The spleen was pulled through the wound, the gastrosplenic omentum was

doubly ligated, divided, and the spleen delivered. The abdomen was closed with interrupted catgut and the rabbits given intramuscular penicillin and streptomycin. Laparotomies (sham splenectomies) were carried out on the five control animals.

Titration of Isoantibodies by Test Tube Agglutination: 0.5 ml. of the modified veronal buffer was pipetted into all the test tubes used in a given titration. Then doubling dilutions of the unknown serum were prepared in the following way: 0.5 ml. of the undiluted serum to be tested was added to the first tube, the contents were mixed, and 0.5 ml. of this mixture was withdrawn and added to the second tube; after mixing, 0.5 ml. of this was added to the third tube, etc. The extra 0.5 ml. from the tube containing the highest dilution was discarded. The entire length of the pipette used was rinsed in buffer between each step. Then 0.2 ml. of a 5 per cent suspension of type GG erythrocytes (from animal #110) in the veronal buffer was added to all the tubes. The reagents were mixed, the tubes randomized and then incubated for one hour at 37° Centigrade. Appropriate negative (buffer rather than unknown serum) and positive (anti-GG typing serum rather than unknown serum) were run with each set of titers. The hemagglutination patterns were recorded as one to four plus by a reader who had no clue to the order of the tubes he was describing.

RESULTS

Results of Blood Typing

Table One

Red Cell Typing of 77 Rabbits with Anti-G and Anti-g Sera

Reaction of Red Cells and Antiserum		Number of Rabbits	Per Cent of Total	Possible Genotype
anti-G	anti-g			
positive	negative	3	3.9	GG
positive	positive	62	80.5	Gg
negative	positive	12	15.5	gg
negative	negative	0	0	--

Table Two

Frequency of Rabbit Blood Groups Reported by Previous Authors

Castle, 1933[41]		Fischer, 1935[42]		Kellner, 1953[39]		Anderson, 1955[43]	
Number of Rabbits Typed							
788		100		215		87	
Blood Group	Incidence (Per Cent)	Blood Group	Incidence (Per Cent)	Blood Group	Incidence (Per Cent)	Blood Group	Incidence (Per Cent)
H_1	28	K_1	32	g	17.7	A	19.3
H_2	44	K_2	19	G	25.5	B	52
H_1H_2	21	K_1K_2	48	gG	57.7	AB	23
0	7	0	1	0	0	0	5.7

Results of Splenectomy

Table Three

Animal Number	Body Weight (grams)	Splenic Weight Immediately Postoperatively (grams)	Per Cent of Body Weight
180	1600	0.750	0.046
148	2500	1.630	0.065
156	1900	1.150	0.079
163	3000	1.080	0.036
71	3000	1.450	0.048

Results of Iso-Immunization

See accompanying figures

The titers for the splenectomized animals were compared with those of the control animals by the use of the Wilcoxon Rank Sum Test.[44] The differences observed between the two groups were no greater than can be satisfactorily explained by chance fluctuation.

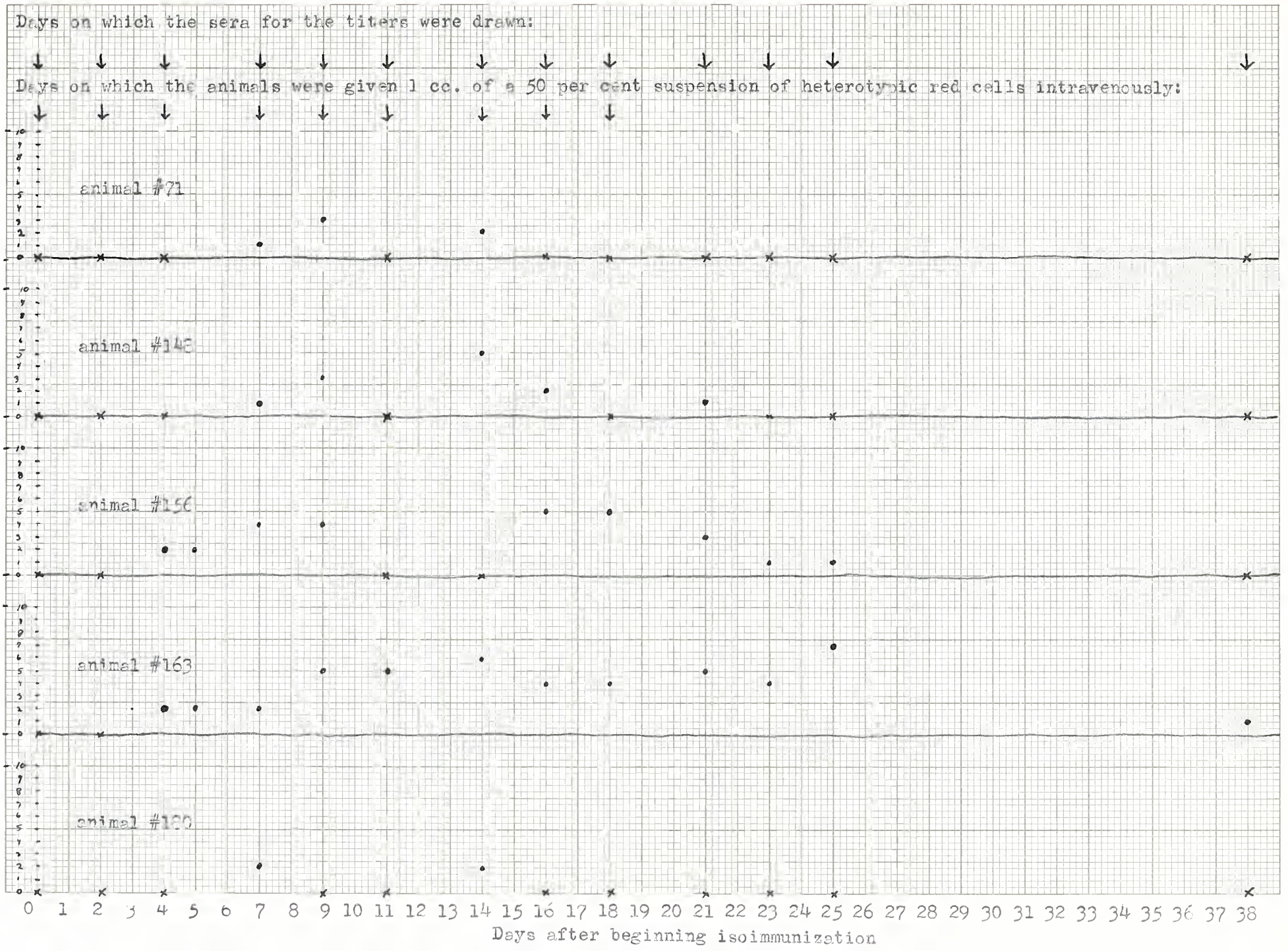

FIGURE ONE: ISOHEMAGGLUTINATION TITERS IN THE SPLENECTOMIZED ANIMALS

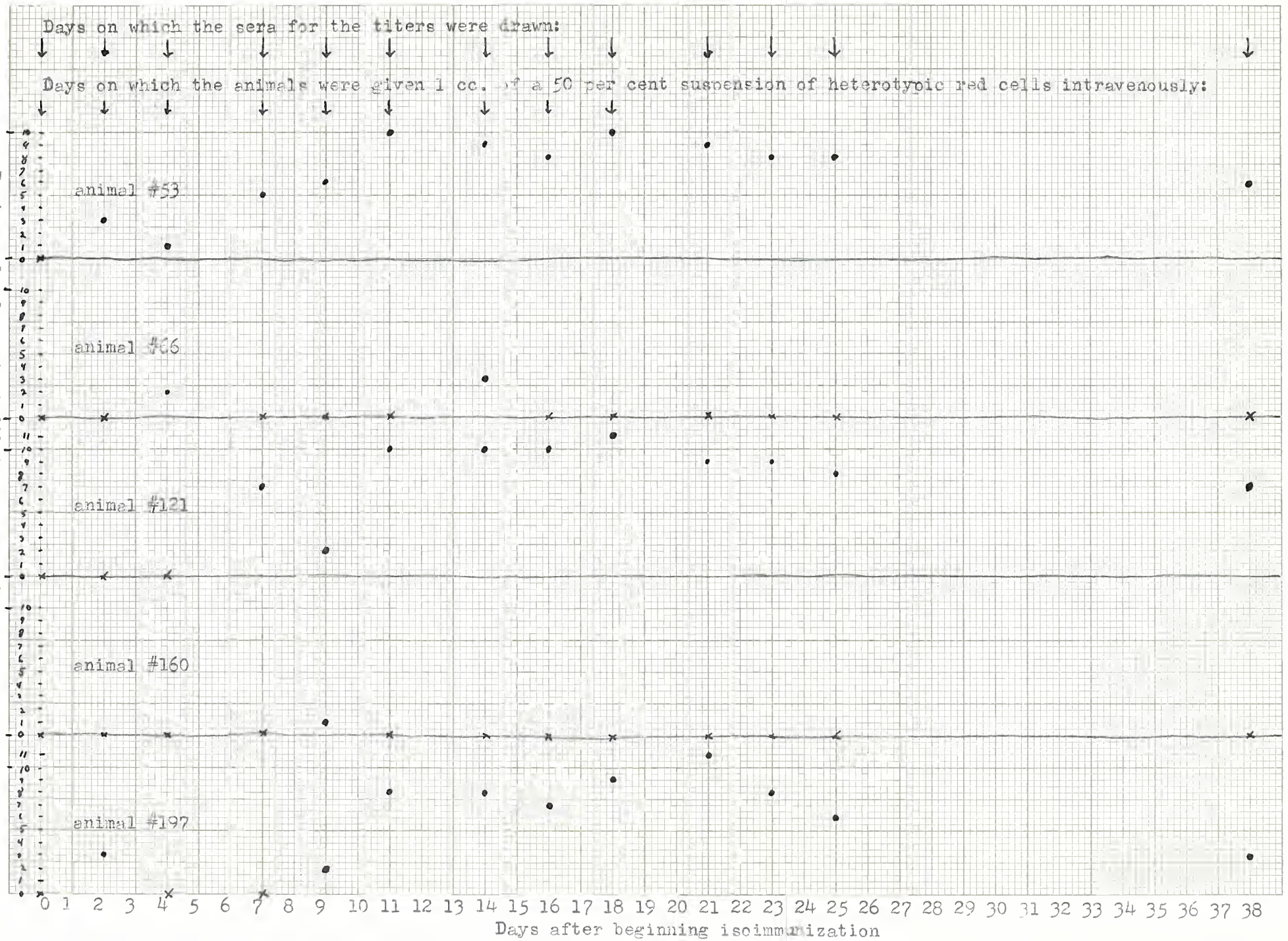

FIGURE TWO: ISOHEMAGGLUTINATION IN THE CONTROL ANIMALS

DISCUSSION

The distribution of blood types in my study differs significantly from those of previous workers (please refer to Tables One and Two), although my typing sera was that used by Kellner and Hedal.[39] Because I sought homozygous subjucts, I blood typed my animals with a simple slide agglutination technique in the field before purchasing them. The other workers used an incubated test-tube agglutination coupled with either titers of trypsin-treated red cells (Anderson)[36] or direct Coombs testing (Kellner and Hedal).[39] Their more sensitive methods may account for some of this variation. However, there is a wide difference in the degree of agglutinability of g-positive cells. Some regularly give strong (four plus) reactions, while others give weak (one plus) reactions, even though the same agglutinating anti-g serum is employed. Such differences appear to be genetically determined[39] and are well known in the A_1-A_2 and the D-Du blood groups in man.[45] Since it is also known that g and G are the same as A and B, it follows that the figures of Anderson[43] and those of Kellner[39](viz. Table Two) cannot both represent the true incidence of these factors in all rabbits, and it seems likely that different strains will show large variation in blood group incidence.

The immunization schedule followed in this research (see accompanying figures) was more successful in stimulating anti-erythrocytic iso-antibodies of high titer than those reported in earlier papers.[34-36] Anderson, using as many as thirty-two 1 ml.

intravenous injections of a 50 per cent red cell suspension had only 15 per cent of his series show titers greater than 1 to 64[36], and Wright used six to ten 1 ml. doses of a 10 per cent suspension, but caused only 15 per cent of his animals to show similarly high titers.[34] Four of my ten rabbits had titers greated than 1 to 64; however, there was no significant difference in response between the control and the splenectomized subjects.

The explanation for this immune response despite splenectomy is most reasonably a complex one and our considerations should include the following:

First: removal of the spleen is probably equivalent to the removal of a large number of the cells of the reticulo-endothelial system and the effect would relate to both the size of the animals total reticulo-endothelial system and the size and composition of its spleen. In the rabbit, the spleen is a small organ (see Table Three), and there is much accessory splenic and hemolymph tissue. Perhaps splenic resection in the rabbit should not be expected to work major changes in antibody response, particularly to a weak antigen like the heterotypic homologous red cell. An adult human spleen averages 0.23 per cent of the body weight (Krumbhaar)[46] three to six times that of a rabbit, a fact that emphasizes the dangers of closely relating human and animal studies.

Second: this was a quantitative study of the isohemagglutinin formation, and we can speculate that splenectomy might induce as yet immeasurable qualitative changes in the gamma

globulins.[47]

Third: there may, in fact, be a depressed response in the splenectomized animals that was not detected in my small study. Statistical machinations reveal that significance would be reached if the sample were doubled, all other factors remaining equal.

SUMMARY

1. Blood typing seventy-seven rabbits revealed notable differences in type frequency from other series. This variation was felt to be compatible with the genetic variations between strains.

2. Splenectomized and control animals were given repeated intravenous injections of heterotypic homologous rabbit red cells. Frequent isohemagglutination titers showed that the isoantibody response of the splenectomized animals was equal to that of the controls. This immune response despite splenectomy was thought due to the small size of the rabbit spleen and the weakness of the rabbit erythrocyte as an isohemagglutinogen.

REFERENCES

1. Perla and Marmorston, Natural Resistance and Clinical Medicine, Little, Brown and Company, 1941.

2. Ehrich and Harris, "The Site of Antibody Formation", Science, vol. 101, 1945.

3. White and Dougherty, "Effect of Prolonged Stimulation of the Adrenal Cortex and of Adrenalectomy on the Numbers of Circulating Erythrocytes and Lymphocytes", Endocrinology, vol. 36, 1945.

4. Bjorneboe and Gormsen, "Plasma Cells as Antibody Procedures, Experimental Studies of the Role of", Acta Pathologica and Microbiologica Scandinavica, vol. 20, 1943.

5. Fagraeus, "The Plasma Cellular Reaction and its Relation to the Formation of Antibodies in Vitro", Journal of Immunology, vol. 58, 1948.

6. Askunas and Humphrey, "Formation of Specific Antibodies and Gamma Globulin in Vitro; A Study of the Synthetic Ability of Various Tissues from Rabbits Immunized by Different Methods", Biochemical Journal, vol. 68, 1958.

7. Rowley, "The Effect of Splenectomy on the Formation of Circulating Antibody in the Adult Male Albino Rat", Journal of Immunology, vol. 64, 1950.

8. Scarff, "Role of Spleen in Antibody Production", Journal of Pathology and Bacteriology, vol. 34, 1931.

9. King and Shumacher, "Splenic Studies: Susceptibility to Infection After Splenectomy Performed in Infancy", Annals of Surgery, vol. 136, 1952.

10. Smith, et al, "Hazard of Severe Infections in Splenectomized Infants and Children", American Journal of Medicine, vol. 22, 1957.

11. Doan, et al, "Hypersplenic Cytopenic Syndromes, A 25 Year Experience with Special Reference to Splenectomy", Proceedings of the Sixth Congress of the International Society of Hematology, Grune and Stratton, 1958.

12. Laski and MacMillan, "Incidence of Infection in Children after Splenectomy", Pediatrics, vol. 24, 1959.

13. Motohashi, "Effect of Splenectomy upon Production of Antibodies", Journal of Medical Research, vol. 43, 1922.

14. Gorden, et al., "The Reticulo-Endothelial System and Hormone Refractoriness", Journal of Experimental Medicine, vol. 70, 1939.

15. Wolfe, et al., "Precipitin Production in Chickens; Effect of Splenectomy on Antibody Formation", Journal of Immunology, vol 64, 1950.

16. Lipp, et al., "Immunisierung non-splenektomierten Kaninchen mit aufgesschlossenem Bakterienantigen und Zellen", Zeitschrift für Immunitätsforschung und Experimentale Therapie, vol. 115, 1958.

17, Nossal, "Studies on the Transfer of Antibody Producing Capacity, 1. The Transfer of Antibody Producing Cells to Young Animals", Immunology (Oxford), vol. 2, 1959.

18. Pfeiffer and Marx, "Untersuchungen über die Bildungsstätte der Choleraantikörper", Deutsche Medicinische Wochenschrift, vol. 24, 1898.

19. Wasserman, "Weiteremittheilungen über Seitenketten-Immunität, Berliner Klinische Wochenschrift, vol. 35, 1898.

20. Castellani,"Ueber das Verhältniss der Agglutinine zu den Schutzkörpern", Zeitschrift für Hygiene und Infectionskrankheiten, vol. 37, 1901.

21. Tsusumi and Kohda, "Ueber die Bildungsstätte des komplement-bindenden Antikörpers", Zeitschrift fur Immunitätsforschung, vol. 19, 1913.

Topley,
22,/"Role of Spleen in Antibody Production", Journal of Pathology and Bacteriology, vol. 33, 1930.

23. Freund, "Distribution of Antibodies in the Serum and Organs of Rabbits,V. The Antibody Content of the Skin at the Site of Injection of Immune Serums", Journal of Immunology, vol. 16, 1929.

24. Pappenheimer, The Nature and Significance of the Antibody Response, Columbia University Press, 1953.

25. Taliaferro and Taliaferro, "Role of Spleen in Hemolysin Production", Federation Proceedings, vol. 9, 1950.

26. Rowley, "The Formation of Circulation Antibody in the Splenectomized Human Being Following Intravenous Injection of Heterologous Erythrocytes", Journal of Immunology, vol. 65, 1950.

27, Levine, et al., "Isoimmunization in Pregnancy: Its Possible Bearing on the Etiology of Erythroblastosis Foetalis", Journal of the American Medical Association, vol. 116, 1941.

28. Bruner, Edwards, et al.,"Icteric Foals", Journal of the American Veterinary Medical Association, vol. 112, 1948.

29. Caroli and Bessis, "Physiopathologie comparée; l'ictère grave des muletons; ses rapports avec la maladie hemolytique du nouveau-né," Semaine de hôpital, Paris, vol. 24, 1948.

30. Young, et al., "Hemolytic Disease in Newborn Dogs", Blood, vol. 6, 1951.

31. Bruner, et al., "Blood Factors and Baby Pig Anemia", Journal of the American Veterinary Medical Association, vol. 115, 1949.

32. Freund and McDermott, "Sensitization to Horse Serum by Means of Adjuvants", Proceedings of the Society of Experimental Biology and Medicine, vol. 49, 1942.

33. Anderson, "The Experimental Production of Erythroblastosis Foetalis in Rabbits", British Journal of Haemotology, vol. 2, 1956.

34. Wright,et al., "Production of Isohemagglutinins in Rabbits for the Determination of Normal Erythrocyte Survival by Differential Hemagglutination", Journal of Immunology, vol. 74, 1955.

35. Cohen, "Stimulatory Effect of BCG on Isoantibody Production in the Rabbit", Proceedings of the Society of Experimental Biology and Medicine, vol. 85, 1954.

36. Anderson, "Production of High Titre Iso-Antibodies in Rabbits by Means of Adjuvants", British Journal of Experimental Pathology, vol. 36, 1955.

37. Address: Dade Reagents, Inc.
1851 Delaware Parkway
Miami, Florida

38. Cohen, "On Blood Groups and Confusion in the Rabbit", Transplantation Bulletin, vol. 5, 1958.

39. Kellner and Hedal, "Erythroblastosis Foetalis in Rabbits", Journal of Experimental Medicine, vol. 97, 1953.

40. Heard, et al., "An Experimental Study of Haemolytic Disease of the Newborn due to Isoimmunization of Pregnancy: An Attempt to Produce the Syndrome in the Rabbit", Journal of Hygiene, vol. 47, 1949.

41. Castle and Keeler, "Blood Groups Inheritance in the Rabbit", Proceedings of the National Academy of Science, vol. 19, 1933.

42. Fischer, "Ueber Blutgruppeneigenschaften beim Kaninchen", Zeitschrift für Immunitätsforschung, vol. 86, 1935.

43. Anderson, "Blood Groups in Rabbits", British Journal of Haematology, vol. 1, 1955.

44. Wilcoxon, "Probability Tables for Individual Comparisons by Ranking Methods," Biometrics, vol. 3, 1947.

45. Race, et al., "Blood Groups of Latvians, A_1A_2BO, MN, and Rh", Annals of Eugenics, vol. 14, 1948.

46. Krumbhaar and Lippincott, "The Post-mortem Weight of the 'Normal' Human Spleen at Different Ages", American Journal of Medical Science, vol. 197, 1939.

47. Gofstein and Gellis, "Splenectomy in Infancy and Childhood", American Journal of the Diseases of Children, vol. 91, 1956.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

_____

_____

NAME AND ADDRESS DATE